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1. Your reference — P31283-/JDU/BOU

2. Patent application number *(The Patent Office will fill in this part)* — 0216371.5 — 13 JUL 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Rowett Research Institute
Greenburn Road
Bucksburn
Aberdeen
AB21 9SB

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention — "Compounds"

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Murgitroyd & Company
165-169 Scotland Street
GLASGOW
G5 8PL

Patents ADP number *(if you know it)* — 1198013

	Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
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	Number of earlier application	Date of filing *(day / month / year)*
7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application		

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

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Claim *(s)*	-
Abstract	-
Drawing *(s)*	4 + 4

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Translations of priority documents	-
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	-
Request for preliminary examination and search *(Patents Form 9/77)*	-
Request for substantive examination *(Patents Form 10/77)*	-
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11. I/We request the grant of a patent on the basis of this application.

Signature Murgitroyd & Co. Date 12 July 2002

Murgitroyd & Company

12. Name and daytime telephone number of person to contact in the United Kingdom: 0141 307 8400

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Compounds

The present invention relates to new analogues of phytochemicals, to compositions comprising these analogues and to the use of these analogues as therapeutic agents.

Particularly but not exclusively the present invention relates to new analogues of flavonoids having improved lipid solubility and the ability to orientate themselves within lipid membranes.

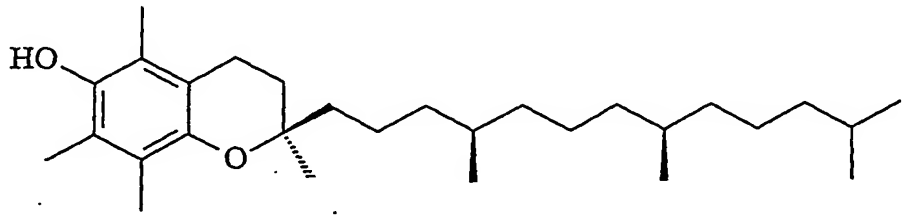
Oxidative damage to cells is implicated in the development of many clinical conditions including ischaemia-reperfusion injury, cancers, heart disease, arthritis, neurological disorders and auto-immune diseases. To date preventative therapy with antioxidants has not been very successful, partly because targeting and orientating the compounds at the correct site within the cell for optimum effect is difficult. Evidence is now emerging that effective antioxidant intervention during the acute

phase of ischaemic events may increase survival rate and minimise irreversible organ damage.

Combinational therapies for treatment of diseases currently incorporate natural and synthetic antioxidants with limited success. There is a need to produce antioxidant agents that possess low toxicity and high therapeutic benefit for use in pharmaceutical preparations. Current natural flavonoid antioxidants are relatively ineffective, being inefficient at targeting molecules.

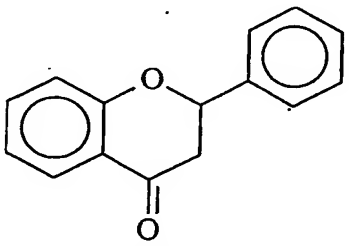
The low bioavailability and uptake by the human body of dietary antioxidants is a limiting factor in their therapeutic action. Dietary antioxidants have poor performance in the treatment of diseases such as Parkinson's and Alzheimer's and in ameliorating ischaemia-reperfusion injury.

Vitamin E (d-α-tocopherol) is a widely used and naturally occurring antioxidant. It is known to protect cell membranes from free radical mediated oxidative damage. The chemical structure of vitamin E (d-(2*R*,4'*R*,8'*R*)-α-Tocopherol), is shown below;

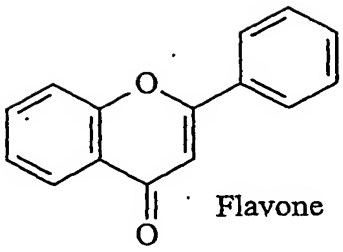


The recognised essential dietary antioxidants are vitamin E and vitamin C. There are also a range of metals; including selenium, iron, copper, zinc and manganese, required from the diet to allow functioning the enzymes with antioxidant activities. Carotenoids from the diet may also have antioxidant properties *in-vivo* in the scavenging of singlet oxygen and in tissues of low partial oxygen pressure.

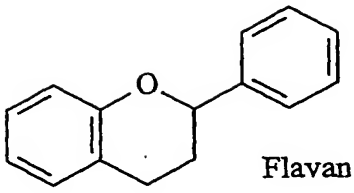
Alternative natural antioxidants include flavonoids which have the following general structure:



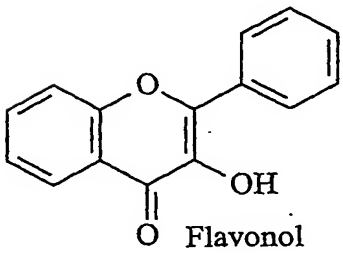
Flavonoids are polyhydroxyphenolic products of the phenylpropanoid biosynthetic pathway in plants, and there are more than 4000 naturally-occurring flavonoids. They are present in a wide range of fruits, vegetables, nuts, and beverages including wine and tea. Flavonoids fall into two distinct groups depending on whether the central heterocyclic ring is saturated or unsaturated. If the central heterocyclic ring is unsaturated (as in anthocyanidin, flavones, flavonols), the molecule is achiral. If the central heterocyclic ring is saturated, as shown above, (as in flavanones and flavans), one or more chiral centres are present, and thus such flavonoids exhibit optical activity. A number of flavonoid structures are shown below;



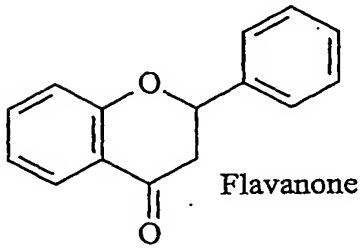
Flavone



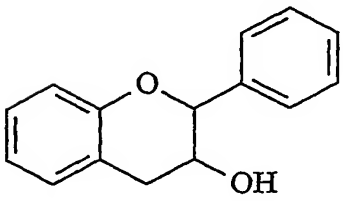
Flavan



Flavonol



Flavanone



Flavan-3-ol

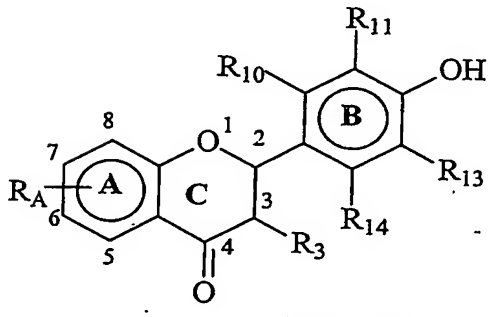
Selected flavonoids, such as myricetin, exhibit potent antioxidant properties and are more effective as antioxidants than vitamin E both in terms of the number of radicals which one molecule can reduce and in terms of the rate of the radical annihilation reaction. However, flavonoids are poor membrane protectants due to their limited lipid solubility. Consequently flavonoids have had limited application as antioxidants *in vivo*.

Our kinetic and stoichiometric studies comparing the reducing capabilities of flavonoids to a d-α-tocopherol indicate that the antioxidant activity is

markedly influenced by the number and position of the hydroxyl groups on the B and C rings as well as the extent of conjugation between the B and C rings. Moreover, within a biological system where a number of polyphenols may be present at similar concentrations, antioxidant efficacy may be predominantly governed by reaction kinetics rather than stoichiometry.

The present invention provides novel compounds having both potent antioxidant activity together with high lipid solubility, thus facilitating their sequestration into the cell membrane.

According to one aspect of the present invention there is provided a compound comprising a group R_A attached to the A ring of a flavonoid group of the following formula I:



Formula I

wherein;

R_A is a C_5 to C_{30} aliphatic alkyl chain; R_{10}, R_{11}, R_{13}, R_{14}, and R_3 each independently represent H, OH, or a C_1 to C_4 aliphatic alkyl; and

optionally there is a double bond between C_2 and C_3 of the C ring.

Preferably at least one of R_{10}, R_{11} and R_{13} represents OH. More preferably R_{11} represents OH.

Suitably both R_{11} and R_{13} represent OH.

Preferably at least three of R_{10}, R_{11}, R_{13}, R_{14} and R_3 represent OH.

Advantageously the flavonoid group is an extended conjugated π-electron system.

Preferably there is a double bond between C_2 and C_3 of the C ring.

Preferably the B and C rings of the flavonoid have the structure of the B and C rings of myricetin, morin, quercetin, kaempferol, luteolin, or apigenin. More preferably the B and C rings of the flavonoid group have the structure of the B and C rings of myricetin.

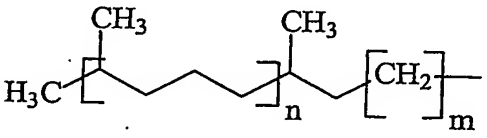
Alternatively the B and C rings of the flavonoid group may have the structure of the B and C rings of taxifolin or catechin.

R_A comprises an alkyl aliphatic backbone of from 5 to 30 carbon atoms. The backbone may be substituted with small alkyl groups, such as CH_3 or C_2H_5. Preferably the backbone of R_A has from five to

twenty carbon atoms, more preferably from eight to fifteen carbon atoms. The backbone may be saturated or unsaturated. Preferably the backbone is saturated.

Suitably R_A is attached to position 5, 6, 7 or 8 of the A ring of the flavonoid group. Preferably R_A is attached to position 7 of the A ring of the flavonoid group.

In a preferred embodiment R_A has the following structure:

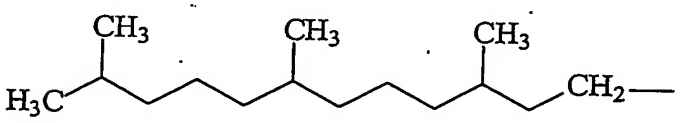


wherein

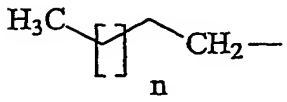
n is an integer from 1 to 7, preferably from 2 to 3;

m is an integer from 1 to 7, preferably from 1 to 2;

More preferably R_A has the following structure:

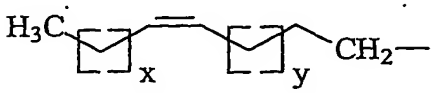


Alternatively R_A has the following structure:



wherein n is an integer from 2 to 27, preferably n is 2 to 17, more preferably 4 to 12.

In another embodiment R_A has the following structure:



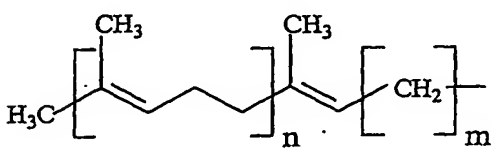
wherein

x is an integer from 1 to 25 preferably 1 to 15, more preferably 2 to 9;

y is an integer from 1 to 25, preferably 1 to 15, more preferably 2 to 9;

and wherein x + y = 25 or less.

~~In another embodiment R_A has the following structure:~~



wherein

n is an integer from 1 to 7, preferably from 1 to 3, more preferably 2;

m is an integer from 1 to 7, preferably from 1 to 3, more preferably 2.

Whilst the Applicant does not wish to be bound by theoretical considerations, it is believed that addition of R_A to the A-ring increases membrane partitioning and also adds the important spatial distribution factor observed with vitamin E. It is anticipated that crossing of the blood/brain barrier will also be enhanced.

According to a further aspect of the present invention there is provided a composition comprising a compound as described above and at least one pharmaceutically acceptable excipient or carrier. The composition may be a sunscreen composition. According to a further aspect of the present invention there is provided a method of preventing UV damage to the skin (for example sunburn or skin cancers such as melanoma) comprising the step of administering a therapeutically effective amount of the sunscreen composition as described above to a patient.

The composition will usually be applied topically.

The composition may alternatively be formulated as a skincare composition and may, for example, include emollients and moisturisers. The skincare

composition may be of particular utility in preventing or reversing the effects of ageing, of reducing apparent wrinkling, and/or treating or preventing dry skin.

According to a further aspect of the present invention there is provided a foodstuff stabiliser composition comprising a compound as described above.

It is believed that the ability to combat free radicals will be of utility in preventing or delaying the deterioration in food quality during storage. It is envisaged that the composition will be particularly effective where the foodstuff stabiliser composition is in the form of an emulsion, especially an emulsion having a low fat/high water content. The foodstuff stabiliser composition will be particularly suitable for low fat spreads, salad dressings etc.

According to a further aspect of the present invention there is provided a method of treating a patient having a disease or disorder involving oxidative damage, said method comprising the step of administering a therapeutically effective amount of the composition described above to said patient.

The disease or disorder involving oxidative damage may be selected from the group consisting of cancer (for example colon, liver or bladder cancer), heart disease, especially to prevent subsequent heart attacks, neurological disorders, (particular mention

may be made of Alzheimer's or Parkinson's disease), auto-immune disorders (particularly arthritis), ischaemia-reperfusion injury, diabetic complications, septic shock, hepatitis, atherosclerosis and complications arising from HIV or Hepatitis B.

Most suitably the disease to be treated is an ischaemia-reperfusion injury.

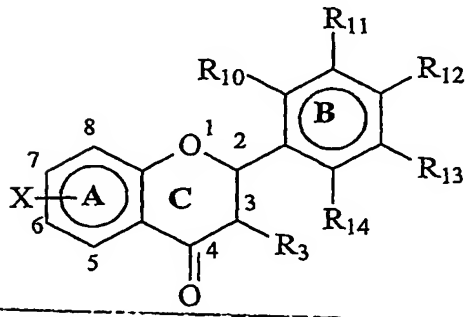
According to a further aspect of the present invention there is provided the use of a compound as described above for the manufacture of a medicament for the treatment of a disease or disorder involving oxidative damage.

Suitably the disease or disorder may be cancer (for example colon, liver or bladder cancer), heart disease, especially to prevent subsequent heart attacks, neurological disorders, (particular mention may be made of Alzheimer's or Parkinson's disease), auto-immune disorders (particularly arthritis), ischaemia-reperfusion injury, diabetic complications, septic shock, hepatitis, atherosclerosis, and complications arising from an immune response to HIV or Hepatitus B. Most suitably the disease or disorder is ischaemia-reperfusion injury.

The composition described above may be used prophylactically or curatively.

According to a further aspect of the present invention there is provided a method of manufacturing a compound as described above, said method comprising providing an intermediate compound A and an intermediate compound B,
wherein intermediate compound A has the structure R_AM wherein M is a metal or metalloid group (such as ZnCl, $SnBu_3$ or MgBr) where the metal is directly attached to R_A, and R_A is a C_5 to C_{30} saturated or unsaturated alkyl chain which may optionally be substituted with small alkyl groups such as CH_3 and C_2H_5 and R_AM is capable of participating in transition metal catalysed cross-coupling reactions;

and intermediate compound B has the following structure:



wherein;
R_{12} represents OH or an O-protecting group
R_3, R_{10}, R_{11}, R_{13}, and R_{14} each independently represent H, OH, a C_1 to C_4 aliphatic alkyl group or an O-protecting group where required, and optionally there is a double bond between C_2 and C_3 of the C ring;

and X is a halogen, O-trifluoromethane sulphonate or any other group used in cross-coupling reactions;

and reacting intermediate compound A with intermediate compound B by transition metal catalysed cross-coupling reactions and subsequently deprotecting at least one OH group.
Preferably R_AM is an organomagnesium, organozinc, organoboron or organotin compound. Alternatively M may be silyl group.

The transition metal catalyst may be any suitable transition metal catalyst used in cross-coupling reaction and particular mention may be made of palladium, nickel or iron complexes.

The protecting group may suitably be methoxymethyl, benzyl (with an optionally substituted aromatic ring) or a small alkyl group such as methyl.

Usually all of the OH groups will be protected but it may be possible that certain groups need not be protected under certain reaction conditions.

The present invention will now be further described by reference to the non-limiting examples and figure in which:

Fig 1 shows the decay curve of the galvinoxyl resonance obtained in ESR timesweep mode (static field) during in situ reduction of the radical by

quercetin. Inset is the fieldsweep spectrum of galvinoxyl.

Example 1

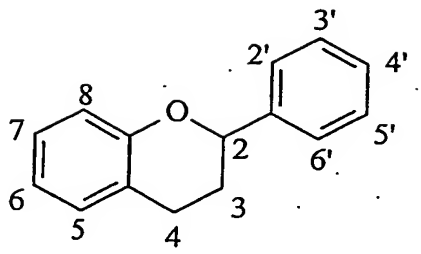
Target 1 (a compound according to the present invention) was synthesised using the reaction shown in Fig 3.

Target 1 retains potent antioxidant activity. This is defined as the reaction stoichiometry between the target and the synthetic free radical, galvinoxyl.

In terms of the physico-chemical properties, the compound partitions overwhelmingly into the organic layer of an octanol-water bi-phasic system. This system is a commonly used model of membrane affinity. Target 1 has a high octanol solubility and a long alkyl chain that should allow optimal orientation in lipid membranes. A unique high-potency and strongly lipid-soluble antioxidant was produced.

The reaction scheme of Example 1 may be repeated using different alkyl chains.

In all the following examples and discussions, we will use the traditional numbering scheme for flavonoids rather than that defined in Formula 1 above. The traditional numbering is as shown below:

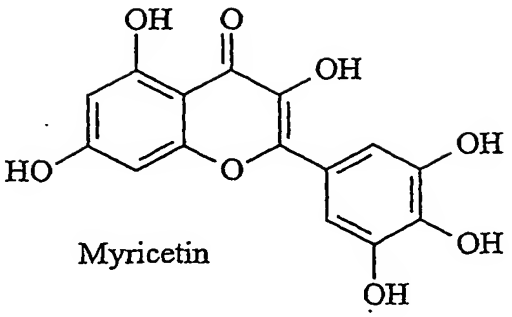


Example 2

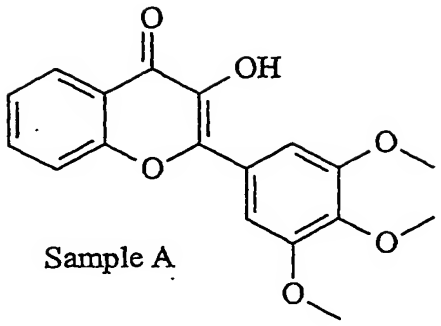
Inhibition of TBARS production in rat liver microsomes from vitamin E-deficient rats by pre-incubation with target antioxidant and related compounds.

Background

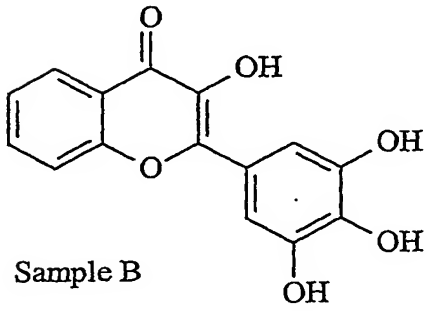
Microsomes are subcellular fractions containing membrane fragments. In vitamin E-deficient rats, microsomes are especially prone to oxidative free radical damage. This can be quantified in terms of the production of thiobarbituric acid reactive substances (TBARS) which result from radical-mediated destruction of the polyunsaturated fatty acid constituents. Consequently, this is a useful biological model to determine the efficacy of phytochemicals as antioxidant membrane protectants. Vitamin E-deficient microsomal suspensions were incubated for 30 minutes with either the target compound (Target 2), myricetin, sample A, sample B, sample C (as shown below) or d alpha-tocopherol.



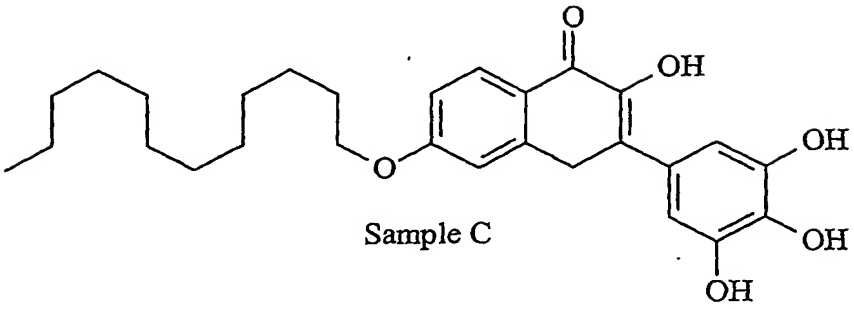
Myricetin



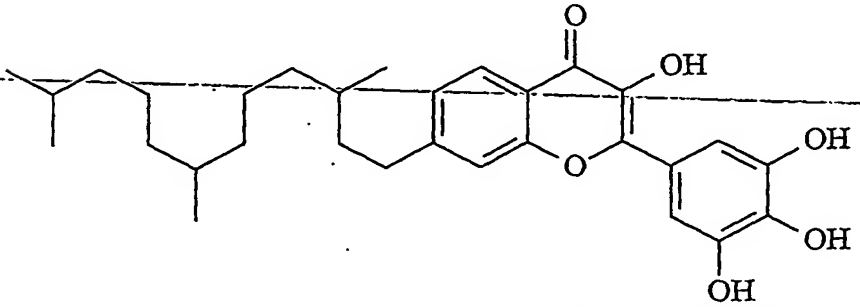
Sample A



Sample B



Sample C



The above Target 2 compound was synthesised using the reaction shown in Figure 4.

The microsomal suspension was then added to solutions containing Fe(II)-ADP/ ascorbate to

initiate free radical-mediated oxidation and incubated for a further 0, 5, 10, 15 or 20 minutes. TBARS production was then measured by HPLC.

Results

In the absence of antioxidant protection (-E), TBARS production increases with time. Myricetin (M), although a potent antioxidant in chemical systems affords almost no protection. Compound A, in which the B-ring substituents are methoxylated is non-protective. We have also demonstrated the lack of antioxidant activity in the ESR chemical model system. Sample B, in which the two hydroxyls of myricetin have been removed to increase lipophilicity, is very soluble in octanol, and we have shown by ESR that it retains potent antioxidant activity. However, it does not give rise to significant membrane protective effects. Sample C, which comprises an unbranched alkyl chain linked to the A-ring *via* oxygen and wherein the alkyl chain length is that of our target molecule, shows efficacy in the initial stages of microsomal oxidation. However, the protection is lost after 20 minutes. The target (T) suppresses oxidative damage throughout the 20 minute period and is comparable in effectiveness to dα-tocopherol (α). The greater efficacy of Target 2 in comparison to sample B shows that orientation within the membrane is vital to suppressing oxidation, and that lipophilicity alone is not sufficient. The greater efficacy of Target 2 in comparison to sample C is probably due to the

methylated chain of Target 2 more closely resembling the side-chain of vitamin E, thus ensuring correct orientation in the membrane.

Example 3

Within a biological system where a number of polyphenols may be present at similar concentrations, antioxidant efficacy may be predominantly governed by reaction kinetics rather than stoichiometry. Consequently, the antioxidant potential of thirteen flavonoids and vitamin E were assessed and their kinetic and stochiometric reduction of a synthetic radical using stopped-flow electron spin resonance (ESR) spectroscopy has been compared. The radical used was galvinoxyl (Galv-O°), (2,6-di-*tert*-butyl-α-(3,5-di-*tert*-butyl-4-oxo-2,5-cyclohexadien-1-ylidene)-*p*-tolyloxy) which is resonance-stabilised and sterically-protected, and so displays little self-reactivity in solution, is reduced by H-atom transfer reactions in the presence of phenolic compounds.

$$\text{Galv-O}^\circ + \text{Phenol-OH} \leftrightharpoons \text{Galv-OH} + \text{phenol-O}^\circ$$

The process is governed by the O-H bond dissociation enthalpy of the donor. Galvinoxyl has a well-defined ESR spectrum and this property was used to calculate second order rate constants, as well as establishing stoichiometry, for the reaction with phenolic compounds.

Materials

Tamarixetin and myricetin-3',4',5'-trimethylether were purchased from Indofine Chemical Co. (Somerville, USA). The remaining flavonoids, d-α-tocopherol and galvinoxyl (2,6-di-*tert*-butyl-a-(3,5-di-*tert*-butyl-4-oxo-2,5-cyclohexadien-1-ylidene)-*p*-tolyloxy) were purchased from Sigma-Aldrich Chemical Co. (Poole, Dorset, UK) and ethanol (>99.7%) from BDH Laboratory Supplies (Poole, Dorset, UK). Reagents were used without further purification.

Methods

Kinetic Measurements

Ethanolic solutions of flavonoid (0.2 mM) and galvinoxyl (0.2 mM) were de-oxygenated under a stream of nitrogen gas. Aliquots (6 ml) were transferred to Hamilton gas-tight syringes (10 ml) coupled to a pneumatic ram and connected to a two-stream ESR quartz flow-cell. *In situ* reaction at 20°C ± 2°C between the flavonoid and galvinoxyl was initiated by rapidly evacuating the syringes. Spectra and decay curves were obtained on a Bruker ECS 106 spectrometer operating at *ca.* 9.5 GHz (X-band) and equipped with a TM_{110} cavity. Decay curves were obtained by operating in timesweep mode with the static field set at the resonance maximum of the galvinoxyl signal.

Stoichiometric Measurements

Ethanolic solutions of flavonoids (0.1 mM) were prepared. Aliquots (3 ml) of an ethanolic galvinoxyl solution (0.5 mM) were mixed with an equal volume of flavonoid solution then transferred to an ESR quartz cell. The spectra and reaction stoichiometry were evaluated. In brief, the spectra of the unreacted galvinoxyl were obtained 5 minutes from mixing, by which time equilibration was complete. The galvinoxyl concentrations remaining were calculated by double integration of the signal and comparing with the control experiment where ethanol was added to the galvinoxyl solution instead of flavonoid solution.

Results

The ESR spectrum of galvinoxyl in an ethanolic solution consists of a doublet of quintets (Figure 1) which arise from the interaction of the unpaired electron spin with the nuclear spins of the proton on the central carbon and the four equivalent aromatic ring protons. In the presence of a hydrogen donating compound, such as quercetin, the resonances decay as reduction of the radical proceeds. Data from all the decay curves gave a good linear fit to the second-order integrated rate expression, with the average correlation coefficient for each set of replicates being greater than 0.970. However, there were marked differences between the flavonoids in the kinetics of the reduction of the

galvinoxyl free radical (Figure 2). Myricetin and morin were, by far, the fastest to react whereas hesperitin and apigenin showed little reactivity. Ranking of reaction rates as second order rate constants was: myricetin > morin > quercetin > fisetin ≈ catechin > kaempferol ≈ luteolin > rutin > taxifolin > tamarixetin > myricetin-3',4',5'-trimethylether > datiscetin > galangin > hesperitin ≈ apigenin. Reaction rates of eight of the flavonoids were greater than that for vitamin E.

The stoichiometry of the reaction of these compounds with the galvinoxyl free radical was determined by adding the flavonoid, or vitamin E, to an excess of the radical and allowing the reaction to proceed to the endpoint. This resulted in a ranking of antioxidant capacity which differed from the kinetic ranking (Figure 2) i.e. myricetin > fisetin > quercetin ≈ luteolin > rutin > catechin > taxifolin > kaempferol ≈ morin > datiscetin > tamarixetin > myricetin-3',4',5'-trimethylether ≈ galangin > hesperitin > apigenin. In particular, the reaction of morin with galvinoxyl had the second fastest rate of all compounds, but was only ranked eighth equal in terms of the number of radicals reduced. Seven of the flavonoids had a greater reaction stoichiometry than vitamin E. Datiscetin, galangin, hesperitin and apigenin were the four lowest ranked of all the compounds in both the kinetic and stoichiometric measurements of antioxidant potential.

Discussion

A large number of natural phenolic compounds in fruit, vegetables, tea and wines have antioxidant activity due to their hydrogen donor activity and their ability to complex transition metal ions. In addition to the location and total number of hydroxyl groups, the solubility of the phenolics in the test medium may significantly affect their ability to act as antioxidants. For example, antioxidant activity of flavonoids in lard appears to be related to the number of *ortho*-dihydroxy groupings in the A and B-rings whereas a lack of conjugation between the B and C-rings is a major influence in aqueous media. The kinetic measurements in the present Application indicate that reactivity of the flavonoids with galvinoxyl in an organic medium is highly-dependent on the configuration of OH groups on the B and C-ring systems.

Galangin, which has no OH groups on the B-ring reacted only very slowly. However, addition of an OH group to the 4′ position (position 12 in formula I) (kaempferol) increased the rate by a factor of about 70. The presence of an OH group on the C-ring was also important because the reaction with apigenin, which has the 4′-OH group (position 12 in formula I), but no OH at the 3-position on the C-ring, was slow, whereas the rate of reaction with kaempferol, which has both of these hydroxyl groups, was almost 250-fold greater.

The importance of further addition of hydroxyl groups to the B-ring was illustrated when comparing luteolin to apigenin. Luteolin is apigenin with an OH added *ortho*- to the 4'-OH (position 12 in formula I). The presence of this catechol function imparts significant activity in its own right as luteolin, which lacks the 3-OH, reacted with galvinoxyl at a rate similar to kaempferol. However, the ability of the 3-OH to enhance reactivity was demonstrated by the doubling of the rate constant in quercetin compared with luteolin.

The difference in rate constant between quercetin and rutin also illustrated the influence that a group at the 3-position has on the kinetics of the reaction of flavonoids with galvinoxyl.

Substitution of the 3-OH of quercetin by an ether-linked sugar group (rutin) caused an approximate 3-fold decrease in the rate of reaction, although the rate constant was still greater than those for apigenin, hesperitin, galangin, datiscetin, taxifolin and vitamin E. By comparison with luteolin, the increased reaction rate of quercetin may be ascribed to electron donation by the 3-OH through the resonance effect, as the B- and C-rings of the flavonoids are linked by an extended, conjugated, π-electron system. In the case of rutin, despite the electron donating ability of the ether group, the rate is lower than that of luteolin. The importance of conjugation is further highlighted by the 7-fold diminution in rate

observed when the C-ring 2,3 bond of quercetin is saturated (taxifolin). More difficult to explain is the activity retained by (+)-catechin which also lacks the 2,3 double bond. Catechin differs from taxifolin by the absence of the C-ring carbonyl group (and use of the single stereoisomer rather than racemic mixture). It may be that the hydrogen of the 3-OH is in close enough proximity to the B-ring to interact and increase the ability of the ring to sustain unpaired electron spin density. Thus a second mechanism to enhance reactivity may operate independent of resonance stabilisation through the 2,3 double bond. With taxifolin, intra-molecular hydrogen bonding of the 3-OH to the carbonyl would inhibit this mechanism and may account for the 5-fold reduction in rate compared with catechin.

Hydroxylation at the 4′ position on the B-ring (position 12 in formula I) was an important feature of reactivity. Comparison of the kaempferol and datiscetin rate constants demonstrated a 56-fold reduction in activity on moving the hydroxyl from the 4′ (position 12 in formula I) to the 2′ position (position 10 in formula I). The presence of a 2′-OH (position 10 in formula I), however, substantially increases the reactivity of a hydroxyl on the 4′ position (position 12 in formula I) as evidenced by the 8-fold increase in rate which morin displays relative to kaempferol. Methoxylation of the 4′-position (position 12 in formula I) of quercetin (tamarixetin) resulted in a 15-fold reduction in

rate suggesting that the O-H bond dissociation enthalpy at the 4′ position (position 12 in formula I) in quercetin is most favourable for H-atom transfer.

Of the fifteen flavonoids examined, eight had rate constants greater than that of vitamin E. Reaction stoichiometries show that many flavonoids can undergo multiple H-atom, or electron transfer, steps (see Table 1). Most effective in this respect was myricetin, in which each molecule could reduce four molecules of the radical. The non-integer values suggest that inter- or intra-molecular side reactions, involving partially-oxidised flavonoid intermediates, occur. The most important determinant of a high stoichiometric value was the presence of a catechol function on the B-ring. Of the fifteen compounds examined, eight were hydroxylated at the 3′ position (position 11 in formula I) and 4′ position (position 12 in formula I) and had reaction stoichiometries ranging from 2.8 (taxifolin) to 4.1 (myricetin). Without this functional group, the highest activity achieved was 1.8 (kaempferol and morin). The enhanced reductive capacity afforded by the catechol moiety is a possible consequence of a two-step oxidation to the *ortho* quinone. Morin, in which the second B-ring hydroxyl group is placed *meta* to the 4′-OH (position 12 in formula I), and consequently is unable to effect quinone formation, has a stoichiometric value of 1.8 compared with 3.3 for quercetin in which the second hydroxyl is placed *ortho* to the 4′ position

(position 12 in formula I). Activity was not a simple function of the number of hydroxyl groups present on the B- and C- rings. For example, datiscetin is morin with the 4'-OH (position 12 in formula I) removed, yet its reaction stoichiometry is essentially the same as that of morin. Rutin, which is quercetin with the 3-OH replaced by an ether-linked sugar moiety, retains similar activity. A poor correlation ($r = 0.44$) was found between the kinetic and stoichiometric parameters for the reduction of galvinoxyl by flavonoids. In particular, datiscetin, kaempferol and morin had almost identical reaction stoichiometries (ca 1.8), yet the reaction rates were 22, 1243 and 10134 mol^{-1} dm^3 s^{-1}, respectively. These results highlight the importance of considering reaction kinetics, as well as stoichiometry, when assessing antioxidant capacity. Where two, or more, potential antioxidants are present, as may occur in complex cellular environments, kinetic factors may greatly over-ride reaction stoichiometry in determining which compound will afford greatest protection. Flavonoids, such as quercetin, may get absorbed from the diet into tissues. Consequently, kinetics and stoichiometry must both be considered in assessing the relevance of plant phenolics as nutritional antioxidants for disease prevention. This ESR method is a useful model to determine these two distinct aspects of antioxidant activity in a non-aqueous environment, as may be encountered in the lipid phase of cells. The galvinoxyl radical is insufficiently oxidising to indiscriminately

abstract H-atoms from a wide range of substrates. Therefore, reactions are only likely to be significant with good H-donors, i.e. compounds which may fulfil an antioxidant role within a biological context.

Compound	k_2	Reaction Stoichiometry	Substitution Pattern							
			3	4	5	7	2'	3'	4'	5'
Catechin	1574±79	2.96±0.01	-H,-OH	-H,-H	-OH	-OH		-OH	-OH	
Taxifolin	337±32	2.82±0.05	-H,-OH	=O	-OH	-OH		-OH	-OH	
Hesperitin	6±0.5	0.20±0.02	-H,-H	=O	-OH	-OH		-OH	-OMe	
Apigenin	5±0.5	0.04±0.02	-H	=O	-OH	-OH			-OH	
Luteolin	1212±45	3.24±0.01	-H	=O	-OH	-OH		-OH	-OH	
Galangin	18±1	1.01±0.03	-OH	=O	-OH	-OH				
Fisetin	1623±199	3.68±0.03	-OH	=O		-OH		-OH	-OH	
Kaempferol	1243±99	1.84±0.01	-OH	=O	-OH	-OH			-OH	
Quercetin	2383±258	3.27±0.04	-OH	=O	-OH	-OH		-OH	-OH	
Tamarixetin	165±20	1.14±0.03	-OH	=O	-OH	-OH		-OH	-OMe	
Rutin	670±41	3.18±0.01	-ORut*	=O	-OH	-OH		-OH	-OH	
Myricetin	14463±1767	4.08±0.01	-OH	=O	-OH	-OH		-OH	-OH	-OH
Tri-Ome-Myricetin	74±14	1.06±0.02	-OH	=O	-OH	-OH		-OMe	-OMe	-OMe
Datiscetin	22±2	1.74±0.02	-OH	=O						
Morin	10134±459	1.83±0.01	-OH	=O	-OH	-OH	-OH		-OH	
Vitamin E	524±48	2.14±0.12			-OH	-OH	-OH			

Second order rate constants (k_2)and reaction stoichiometries for the reduciton of galvinoxyl radical by flavonoids and vitamin E. *Rutin is quercetin-3-rutinoside. The compounds above the dotted line are based on the 2-H flavan system, while those below are Δ-2-flavan-4-ones.

Figure 1

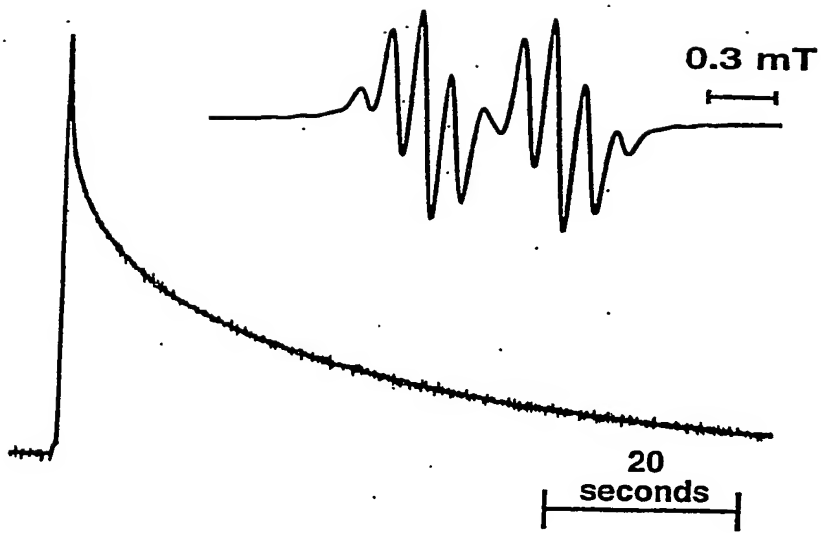
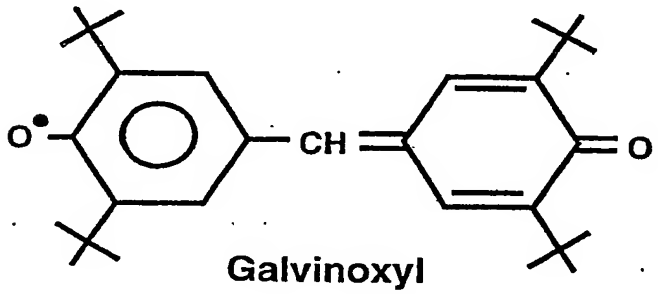
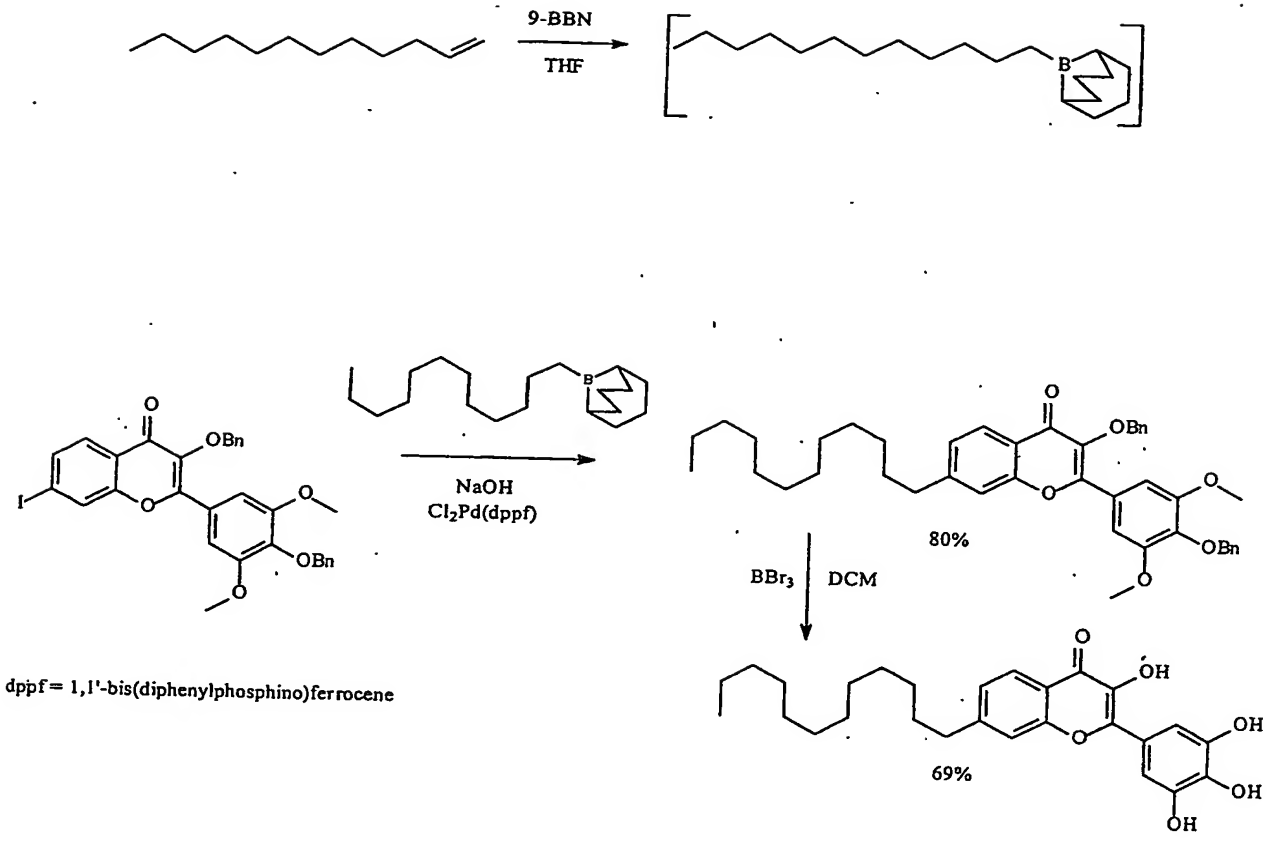


Figure 2

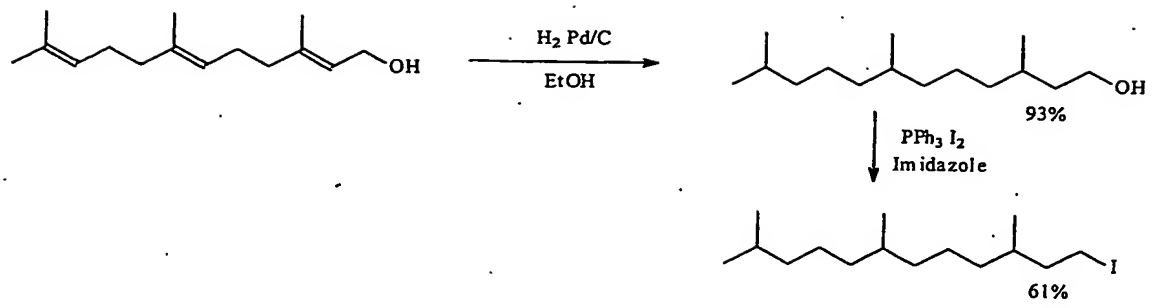


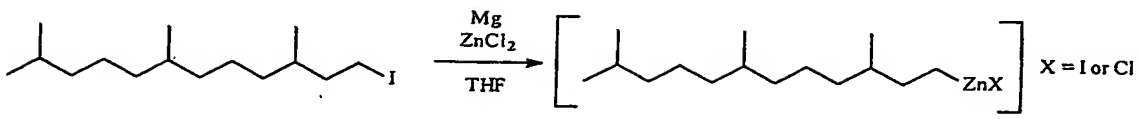
Galvinoxyl

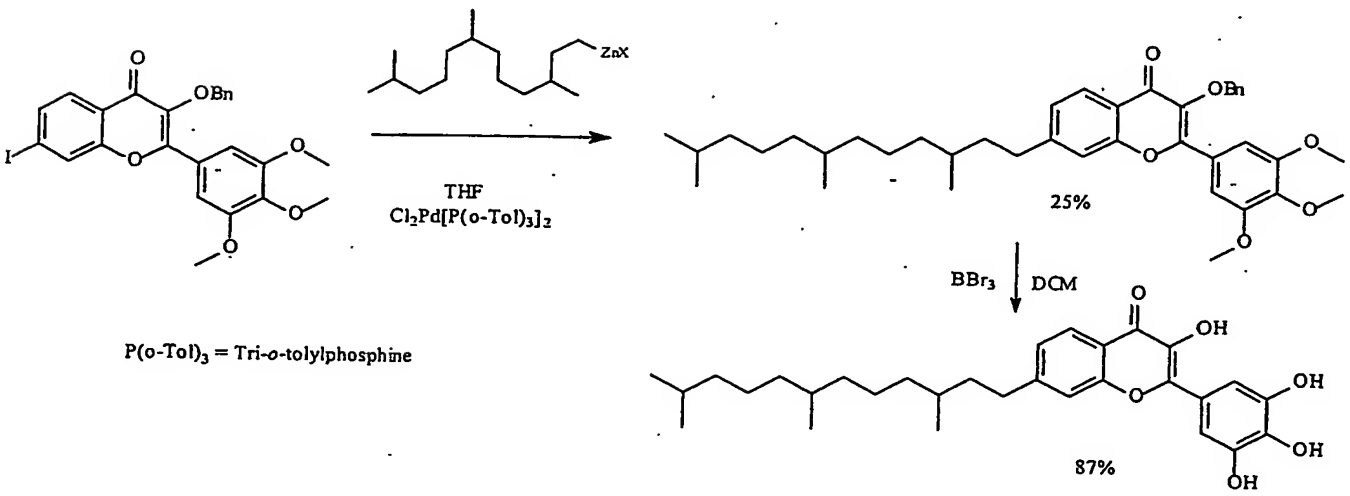


dppf = 1,1'-bis(diphenylphosphino)ferrocene

Fig 3







P(o-Tol)$_3$ = Tri-*o*-tolylphosphine

Fig 4

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